Alteration of Molecular Neurochemistry: MRI-guided Delivery of Viral Vectors to the Primate Amygdala

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Target Audience

Clinician scientists in neurology, neuroradiology, and psychiatry; imaging scientists interested in interventional MRI.

Purpose

Despite current treatments many psychiatric patients remain refractory and suffer from chronic and debilitating diseases. Because of these patients' extreme suffering, invasive treatment methods such as deep brain stimulation for Major Depressive Disorder are being investigated. Progress in understanding the genetic, neurochemical, and molecular bases of these illnesses has made possible the promising approach of using viral vectors to deliver novel therapeutics to relevant neural circuits. We are pioneering strategies that ultimately aim to treat anxiety and other affective disorders through precise delivery of viral vectors to the central nucleus of the amygdala (CeA), a critical output structure coordinating the behavioral and neuroendocrine components of fear and anxiety [1,2]. We present an MR guidance approach for a precise therapeutic delivery, first refined in non-human primate (NHP) models and now being used routinely to deliver viral vectors containing constructs aimed at altering the expression of anxiety in developing nonhuman primates.

Methods

MR-guidance of drug distribution for psychiatric applications is valuable for these reasons: 1) insensitivity to brain shift when targeting small structures, 2) ability to monitor much smaller infusion volumes than those used in more common convection-enhanced delivery (CED) infusions and 3) value in mechanistic studies for focal treatments. The NHP brain is approximately 1/9 the volume of a human brain with the CeA occupying a cylindrical shaped volume of approximately 2 x 2 x 5 mm, with the long axis oriented through plane in Fig. 1. White matter tracts only 3 mm superior and lateral to the desired structure are undesired potential escape paths for CED infusions. As this study is attempting to correlate observable behavioral changes with functional alterations to specific brain regions the necessity for focal treatments is much greater than in other CED studies, such as those with neuroprotective agents that can be used to overtreat because of their strong safety profiles. Our design aims to focus treatment within the CeA region through use of two catheter placements, one of which is visible in Fig. 1, distributed along the longer anterior-posterior axis of the CeA. To minimize infection in surrounding structures infusion volumes are limited to 12 µl per catheter insertion.

All surgical procedures are performed under isofluorane anesthesia following strict guidelines for animal care and with the approval of the local IACUC. Each rhesus monkey is placed in an MRI-compatible stereotactic frame in a GE Healthcare MR750 3.0 T scanner (GE Healthcare; Waukesha, WI). Prior to the procedure, a bilateral craniotomy is performed and two Navigus pivot-point based aiming bases (Medtronics, Inc.; Minneapolis, MN) are installed on the skull. A 3D T1W IR-Prep GRE scan (0.35x0.35x0.8mm voxel, 6.5min) is used to identify two target points in each hemisphere, chosen in the anterior/medial and posterior/lateral ends of the CeA. Catheters are investigational valve-tip catheters (Engineering Resources Group (ERG); Hialeah, FL) and infusions of $3.1x10^{13}$ GC/mL AAV2 viral vector (coded to promote corticotropin-releasing factor (CRF) production) mixed with 0.66 mM gadobenate (MultiHance) are delivered at 1 µL/min for 12 min per target using a PHD 22/2000 constant rate infusion pump (Harvard Apparatus; Holliston, MA) and real-time pressure monitoring system (ERG).

Our previously described targeting system[3] is used to align and insert the catheter. A 2D T1W high resolution scan is continuously collected along the axis of the catheter during infusion and a 3D IR-Prep GRE image is collected post-infusion to visualize the drug distribution. This process is performed four times in sequence, twice on each side of the brain.

We optimized delivery during four protocol refinement surgeries followed by necropsy correlations in three of the four cases, including detection of protein expression in one study. The first 5 of 24 NHP survival subjects have completed surgery and are now part of a three year observation period to assess efficacy of the treatment. Protein expression data are not yet available.

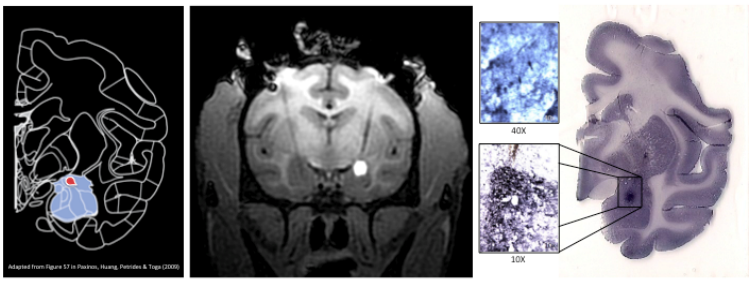

Figure 1: Precise viral vector delivery to the dorsal amygdala: **Left:** Rhesus monkey atlas through the coronal plane demonstrates heterogeneous nature of the amygdala region (purple). The target volume is in the central nucleus region of the amygdala highlighted in red. **Center:** Hyperintense region validates vector delivery to CeA. **Right:** CRF expression at the injection site. Magnification of CeA validates precise neuronal delivery (10X bottom, 40X top)

Results and Discussion

To date we have performed 2-4 infusions in each of the initial 4 subjects, and 4 infusions each of the following 5 subjects at typical depths of 30 mm. In one subject, a Gd tracer was utilized only on one side of a bilateral experiment with identical infusion protocols as shown in Fig. 1. A histological comparison confirms that the low doses Gd tracer (0.66 mM) did not alter the distribution of viral vector expression[4] as shown in Fig. 1. After the refinement studies, intraoperative guidance and monitoring using co-infused Gd indicate proper viral vector distribution in the five longitudinal assessment subjects. Analysis of the infusion centroid demonstrated catheter positioning with sub-mm accuracy. A protocol for stylet extension was adopted to minimize catheter occlusions and prevent creation of tissue voids distal to the catheter tip. In general, given the small target and infusion volume, accurate targeting of the infusion requires considerably more care relative to use of CED for chemotherapy or neuoroprotective agents.

Conclusion

An MR image-guided approach is essential for administering and monitoring the extremely focal delivery of promising agents currently being tested to directly and discreetly alter molecular neurochemistry to treat psychiatric disease.

References

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